Autologous haematopoietic stem cell transplantation for treatment of multiple sclerosis

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Abstract | Autologous haematopoietic stem cell transplantation (AHSCT) is a multistep procedure that enables destruction of the immune system and its reconstitution from haematopoietic stem cells. Originally developed for the treatment of haematological malignancies, the procedure has been adapted for the treatment of severe immune-mediated disorders. Results from ~20 years of research make a compelling case for selective use of AHSCT in patients with highly active multiple sclerosis (MS), and for controlled trials. Immunological studies support the notion that AHSCT causes qualitative immune resetting, and have provided insight into the mechanisms that might underlie the powerful treatment effects that last well beyond recovery of immune cell numbers. Indeed, studies have demonstrated that AHSCT can entirely suppress MS disease activity for 4–5 years in 70–80% of patients, a rate that is higher than those achieved with any other therapies for MS. Treatment-related mortality, which was 3.6% in studies before 2005, has decreased to 0.3% in studies since 2005. Current evidence indicates that the patients who are most likely to benefit from and tolerate AHSCT are young, ambulatory and have inflammatory MS activity. Clinical trials are required to rigorously test the efficacy, safety and cost-effectiveness of AHSCT against highly active MS drugs.

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Ablation of the immune system followed by autologous haematopoietic stem cell transplantation (AHSCT) for the treatment of multiple sclerosis (MS) has been explored for approximately two decades, since the original, pivotal report of its feasibility[1]. Studies have demonstrated that AHSCT has a high efficacy for the suppression of inflammatory MS activity (clinical relapses and activity detectable with MRI)[2,3] and have provided evidence that the procedure leads to neurological improvement in patients with relapsing–remitting MS (RRMS)[4]. AHSCT-related mortality was initially high and deterred referrals for the treatment (which was offered either in trials or compassionately) but has decreased over the past decade. The field has been strengthened by mechanistic studies that have demonstrated that so-called immune resetting underlies the anti-inflammatory effects of the treatment[5–8] and by the publication of clinical studies in the past 2 years that have demonstrated considerable efficacy and acceptable safety[9–14]. Furthermore, mass media has repeatedly featured AHSCT, raising public interest but also inducing some unrealistic expectations among patients with MS and their families, as well as causing confusion among clinicians who are inexperienced in this treatment.

In this Review, we aim to provide a clear and informative description of the treatment procedure for AHSCT, and an overview of current knowledge and outstanding questions about the mechanism of action of the treatment. We then present an up-to-date critical analysis of the published evidence on the efficacy and risks of AHSCT, and give our expert opinion on optimal patient selection and treatment methodology. We also discuss further research required to optimally define the safety, efficacy and cost-saving opportunities of AHSCT compared with currently approved therapies for MS.

Unmet needs in MS therapy

MS affects over 2.3 million people worldwide and is the most common nontraumatic cause of disability in young adults. Besides the incalculable effect on individuals' quality of life, the annual fiscal cost of MS in Europe has been estimated at €14.6 billion[15]. Nevertheless, the development of treatments for MS has been a success story since the 1990s, when the first therapeutics were licensed for the treatment of RRMS. The introduction of the number of brain lesions detected with MRI as an outcome in phase II trials in RRMS has accelerated

Key points

- Ablative therapy and autologous haematopoietic stem cell transplantation (AHSCT) is an increasingly studied and used strategy for the treatment of multiple sclerosis (MS)
- AHSCT confers benefits for patients with MS by achieving radical suppression of inflammatory MS activity
- Qualitative changes in the reconstituted immune system and durable remissions without additional immune intervention support the notion that AHSCT regenerates the immune system (a process known as immune resetting)
- Complete suppression of MS disease activity for 4–5 years has been documented in 70–80% of patients with relapsing–remitting MS who have undergone AHSCT; neurological improvements have also been demonstrated
- Optimal candidates for AHSCT are young, ambulatory and have inflammatory-active relapsing–remitting MS (RRMS); current appropriate indications for AHSCT include aggressive and highly active treatment-refractory RRMS
- Clinical trials to compare AHSCT with approved drugs in RRMS and determine its benefits in inflammatory-active progressive MS are warranted, but progress is hindered by a lack of investment and funding

the licensing of new therapies owing to its excellent association with relapses in trials of up to 3 years[16]. At least ten disease-modifying treatments are now available for RRMS. Treatment development in RRMS has been characterized by phase II trials in which the MRI markers of disease activity have been targeted, and phase III trials of up to 3 years in which clinical relapses and/or evolution of disability have been targeted. However, trials have not demonstrated consistent effects on disability progression, which has the greatest impact on the lives of people with MS. In addition, little evidence is available to inform the optimal choice of disease-modifying treatment for individual patients or when to stop or change therapy. Opinions and practices are split between escalation and induction approaches. In escalation therapy, the safest agent — which is not necessarily the most effective — is tried first, and more-active agents, which usually entail higher risks of adverse effects, are only used if disease activity persists or breaks through. Induction therapy aims to stop or cure the condition before it produces major adverse effects, although it might be associated with higher risks. Alemtuzumab is the licensed therapy that most closely meets the definition of an induction therapy, but is used more frequently after failure of first-line agents.

Besides uncertainty about optimal treatment strategies for RRMS, clear unmet needs exist for effective disease-modifying treatment in certain subgroups of MS: aggressive MS, treatment-refractory MS and progressive MS (BOX 1). These non-mutually exclusive groups can occur at different stages in the natural history of MS, but are all thought to be associated with a worse prognosis than mild to moderate RRMS that responds adequately to disease-modifying treatment.

AHSCT is a one-off treatment designed to eradicate or induce long-term suppression of MS, and could meet the definition of an ideal induction therapy that could be used to treat even the most aggressive disease. To date, however, AHSCT has predominantly been employed as a rescue therapy after an escalation sequence in which more than one line of treatment has failed.

Leukoapheresis
A process that separates white blood cells from the peripheral blood, which, in the context of AHSCT for MS, is carried out with a semi-automated medical device to harvest the patient's autologous haematopoietic stem cell-enriched blood product after mobilization.

Bone marrow aplasia
A state in which the bone marrow fails to generate adequate numbers of haematopoietic stem cells to repopulate the blood with red blood cells, white blood cells and platelets; in the context of AHSCT for MS, this state follows the conditioning regimen, irreversibly after myeloablative conditioning, which necessitates haematopoietic stem cell support for survival

Fundamentals of AHSCT

Haematopoietic stem cell transplantation is a well-established multistep procedure designed to replace the blood and lymphoid systems of a patient with a new one derived from haematopoietic stem cells (HSCs). HSCs can be collected from either a healthy donor (allogeneic transplantation) or from the patient (autologous transplantation). The procedure has been used extensively in the past 50 years for the treatment of aggressive haematological malignancies, such as leukaemia and lymphoma[17]. Allogeneic transplantation is most frequently used for malignant indications, but carries the risk of graft-versus-host disease (GVHD), which increases transplant-related mortality. The risk is partially offset by a lower incidence of leukaemia relapse than that observed with autologous transplantation, an advantage that is attributed to a graft-versus-leukaemia effect. The prevention of relapse by means of graft-versus-host effects has also been reported in autoimmune disease (graft-versus-autoimmunity)[18], but the risk of transplant-related mortality from allogeneic transplantation is generally considered unacceptable in non-neoplastic diseases. Consequently, only autologous transplantation is being developed for the treatment of MS[19].

The procedure

The AHSCT procedure comprises four main steps: HSC mobilization, HSC harvesting, ablative conditioning and HSC re-infusion or 'transplantation' (FIG. 1). Initially, HSCs were obtained by aspiration of the bone marrow, but are increasingly harvested from peripheral blood after so-called mobilization. HSC mobilization involves administration of granulocyte colony-stimulating factor (G-CSF), either alone or with cytotoxic chemotherapy, such as cyclophosphamide. HSCs that have been mobilized are then harvested from peripheral blood by leukoapheresis. The HSCs are cryopreserved and stored frozen until the patient is ready for transplantation. Before transplantation, ablation of the haemato-lymphopoietic system is achieved with high-dose chemotherapy (or chemoradiotherapy when associated with total body irradiation, which is no longer used for MS but is for other indications); this stage is known as the preparative or conditioning regimen. Immediately after completion of the conditioning regimen, patients develop pancytopaenia and a transient bone marrow aplasia, and intravenous infusion of the stored HSCs (transplantation) is required to enable marrow repopulation, recovery of haematopoiesis, and immune reconstitution.

The duration of HSC mobilization and leukoapharesis is 5–15 days, depending on the protocols employed, and can be performed in day care or with a short hospital admission. Conditioning and transplantation require hospital inpatient admission to enable close monitoring and supportive care. Ablative conditioning therapy generally starts at least 2–4 weeks after completion of HSC harvesting, but should not be delayed if it is safe to proceed. Patients are usually admitted for 3 weeks[20].

Pattern II MS pathology
One of four described patterns of tissue pathology in MS, characterized by anti-myelin antibodies and complement factors

T cell receptor excision circles
Episomal DNA circles that are by-products of intra-thymic T cell receptor rearrangement and persist in T cells as detectable markers of their recent thymic origin.

Recent thymic emigrants
T cells that have recently emerged from the thymus after differentiation and thymic selection.

Mechanisms of action

Rationale. Immune-related genetics are important in the aetiology of MS: the HLA-DR15 haplotype is a major risk factor for MS, and >100 common genetic variants — which are enriched for immunologically relevant genes — are associated with the disease[21]. However, genetics explain only a small amount of the risk of developing MS. Several environmental risk factors, including Epstein–Barr virus infection[22], low vitamin D3 levels[23], smoking[24] and obesity[25], have been identified. Events that can initiate MS have not yet been identified, but current evidence suggests that the process involves aberrant activation or failed regulation of proinflammatory T cells, including $CD4^+$ T_H cells that secrete IFNγ (T_H1 cells), IFNγ and IL-17 (T_H1^* cells), and IL-17 alone (T_H17 cells). In pattern II MS pathology[26], a key role for T_H2 cells[27] has been identified. $CD8^+$ T cytotoxic cells, B cells and macrophages are also thought to contribute to immune mechanisms in MS. Detailed information about the immunopathological process is available elsewhere[28,29]. On this basis, the application of AHSCT to MS is intended to eliminate the aberrant adaptive immune system via the conditioning regimen and subsequently rebuild the immune system in the hope that immune tolerance will be re-established.

Immune resetting. Studies from the past decade have shown that rebuilding of the immune system is indeed possible. Immediately after the AHSCT procedure, a broad spectrum of lymphoid and myeloid cells is either completely eliminated or depleted, depending on the intensity of the conditioning regimen. These cells include adaptive immune cells (T cells and B cells) and innate immune cells (natural killer cells, dendritic cells, monocytes and granulocytes). Subsequently a 'new' immune system gradually develops from the $CD34^+$ haematopoietic stem cells. Natural killer cells, $CD8^+$ T cells and B cells are repopulated in the first few weeks to 6 months[30–34], whereas reconstitution of the $CD4^+$ repertoire takes up to 2 years[5]. Important questions that have been addressed about this process include how immune cells redevelop in adults with an inactive remnant thymus, and whether the immune repertoire after AHSCT is indeed new, rather than the result of expansion of cells that survive the conditioning or are present in the graft, and possible acquisition of new functional phenotypes by such cells. Studies that have addressed these questions (described below) have enabled a working model to be constructed for the mechanisms of AHCST in MS (FIG. 2).

T cells. On the basis of our understanding of MS pathogenesis, outlined above, the effect of AHSCT on T cells has been the main focus of research into the mechanisms. The first notable observation about T cell repertoire reconstitution was that naive T cells re-emerged over time after AHSCT and showed signs that they had developed in a re-activated thymus[5]. In that study, analysis of the cellular marker CD31 and so-called T cell receptor excision circles, which mark recent thymic emigrants, showed that T cells generated after AHSCT had undergone positive selection and matured in the thymus[5]. Further evidence for true T cell renewal was obtained from DNA sequencing to enable comparison of the complementarity determining region 3 (CDR3) of T cell receptors (TCRs): comparison of CDR3 sequences from $CD4^+$ and $CD8^+$ T cells before and after AHSCT demonstrated not only that the T cell repertoire was substantially more diverse after AHSCT but also that almost all $CD4^+$ T cells that were present before AHSCT had gone, and new clones had developed[5]. A deep-sequencing study that included millions of TCRs confirmed that repertoire renewal is almost complete for $CD4^+$ T cells, but is less so for $CD8^+$ T cells, in which clonal persistence was seen[8]. One phenotypic study has revealed that levels of presumably pathogenic $CD4^+$ T_H17 cells were reduced after AHSCT, whereas the frequencies of T_H1 and T_H2 cells, which have the potential to induce inflammation and antibody production, respectively, in MS, did not change[7]. Transient increases of $CD4^+CD25^{high}FoxP3^+$ T_{reg} cells and of $CD56^{bright}$ natural killer cells (cells with reported immune regulatory activity[28]) were observed after transplantation; moreover, the post-transplantation T cell repertoire was characterized by expansion of $CD8^+CD57^+$ T cells with the potential to kill autologous $CD4^+$ T cells and consequently to curtail T_H cell activities[6]. In the same study, mucosal associated invariant T (MAIT) cells (characterized by expression of CD8, high expression of CD161 and secretion of IL-17 and IFNγ) were observed in active MS lesions in post-mortem brain tissue and in the peripheral blood of patients before therapy, but this candidate inflammatory cell population was significantly more depleted after AHSCT than after conventional treatments.

Box 1 | Subtypes of MS with unmet clinical needs

Aggressive MS
Aggressive multiple sclerosis (MS) represents almost 10% of all MS cases[90]. Definitions are variable, but commonly include frequent relapses, early acquisition of disability (often with incomplete resolution) and highly active disease seen as new Gadolinium-enhancing lesions and/or new T2 lesions on MRI[90]. 'Rapidly evolving severe MS' and 'malignant MS' are other terms used to refer to disease associated with a poor prognosis. We prefer the term 'aggressive MS' over 'malignant MS' (REF. 108) because it evokes a clinical challenge that can be tackled with aggressive therapies, including autologous haematopoietic stem cell transplantation. The term 'rapidly evolving severe MS' was introduced by healthcare authorities, and is defined as relapsing–remitting MS with two or more disabling relapses in the past year, and one or more Gadolinium-enhancing lesion on MRI or an increase in the T2 lesion load from the previous MRI[109]. Although this term has a specific definition, external validation of the criteria is lacking, and we prefer the more generic term of aggressive MS.

Treatment-refractory MS
MS that persists or breaks through despite disease-modifying treatment is referred to as treatment-refractory MS. Currently, this definition implies a failure to respond to a highly active therapy, though it has been used to refer to failure of first-line therapies.

Progressive MS
In aggressive and treatment-refractory MS, the eventual outcome is accumulation of disability, usually measured as persistent increases in Expanded Disability Status Scale (EDSS) scores in trials and, to a variable extent, in clinical practice. Increasing EDSS scores can result from residual damage from relapses or from progressive disease. Progressive disease, defined by gradual worsening of neurological function for at least 6 months with or without superimposed relapses, is associated with neurodegenerative processes that cause axonal loss. However, progression might be driven by inflammation behind the blood–brain barrier[110–112], so targeting this inflammation might ameliorate progression to some extent, as suggested in trials of B-cell targeted therapies[91].

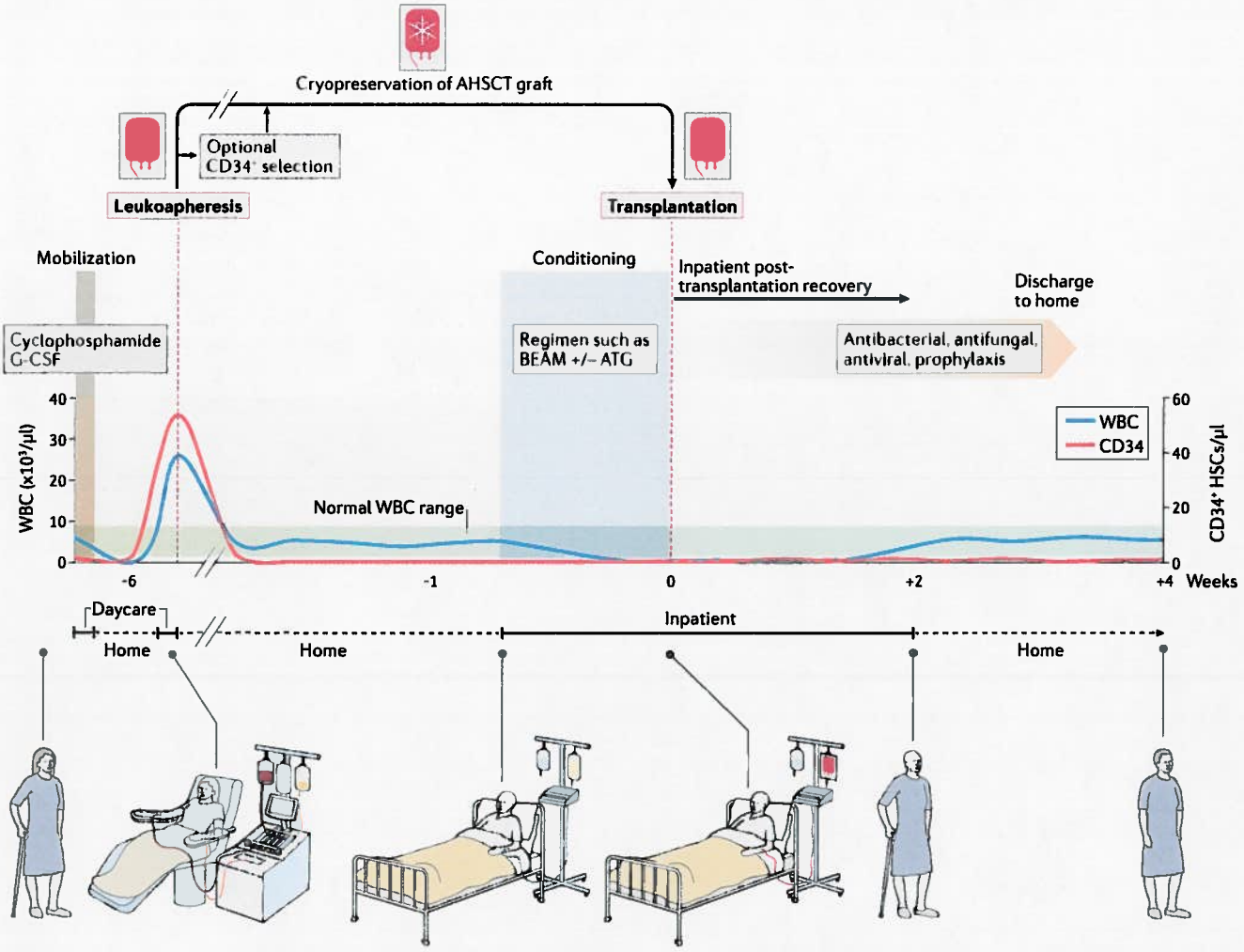

Figure 1 | **Outline of the AHSCT procedure.** Key steps of the procedure, drugs administered and white blood cell (WBC) and CD34+ haematopoietic stem cell (HSC) counts, and the patient's condition and disposition are arranged from top to bottom. The indicative timescale covers 10 weeks. The procedure starts with mobilization of HSCs from the bone marrow by injection of cyclophosphamide intravenously and granulocyte-colony stimulating factor (G-CSF) subcutaneously. The autologous graft harvested from the peripheral blood by leukoapheresis, and which can undergo CD34 selection to enrich HSCs or can be unmanipulated, is cryopreserved for subsequent use. Ablation of the immune and, to a variable extent, myeloid system is most commonly achieved by high-dose conditioning with a combination of cytotoxic drugs. The autologous haematopoietic graft is then reinfused (transplantation), and antithymocyte globulin (ATG) is often administered with the conditioning regimen to deplete T cells; owing to the long half-life of ATG, it will also deplete and prevent the engraftment of any T cells present in the autologous graft (*in vivo* graft T cell depletion). Different levels of supportive care are required during the procedure; the conditioning, transplantation and *in vivo* T cell depletion steps require inpatient admission until the patient has recovered from neutropaenia and the management of any complications is complete. AHSCT, autologous haematopoietic stem cell transplantation; BEAM, bis-chloroethylnitrosourea, etoposide, cytosine arabinoside and melphalan.

Limited data are available about the antigen specificity of T cells after AHSCT. Myelin-specific T cells are known to develop after AHSCT[7,35], but such cells are part of the physiological immune repertoire[36] and more detailed studies of their antigen avidity, functional phenotype and migratory potential are needed to discern whether potentially pathogenic cells redevelop. Evidence from AHSCT for juvenile idiopathic arthritis and dermatomyositis indicates that T_{reg} cells have limited TCR diversity before AHSCT, but are present in greater numbers and are more diverse after AHSCT[37]. Expansion of $CD4^+CD25^{high}FoxP3^+$ T_{reg} cells and increased expression of the regulatory markers CTLA-4 and GITR on $CD4^+CD25^{high}$ cells after AHSCT[38] suggest improved control of T cell activation.

B cells. The effects of AHSCT on B cells and the humoral immune response have been studied less than those on T cells, particularly in MS. Ablative conditioning eliminates B cells at almost every stage of differentiation,

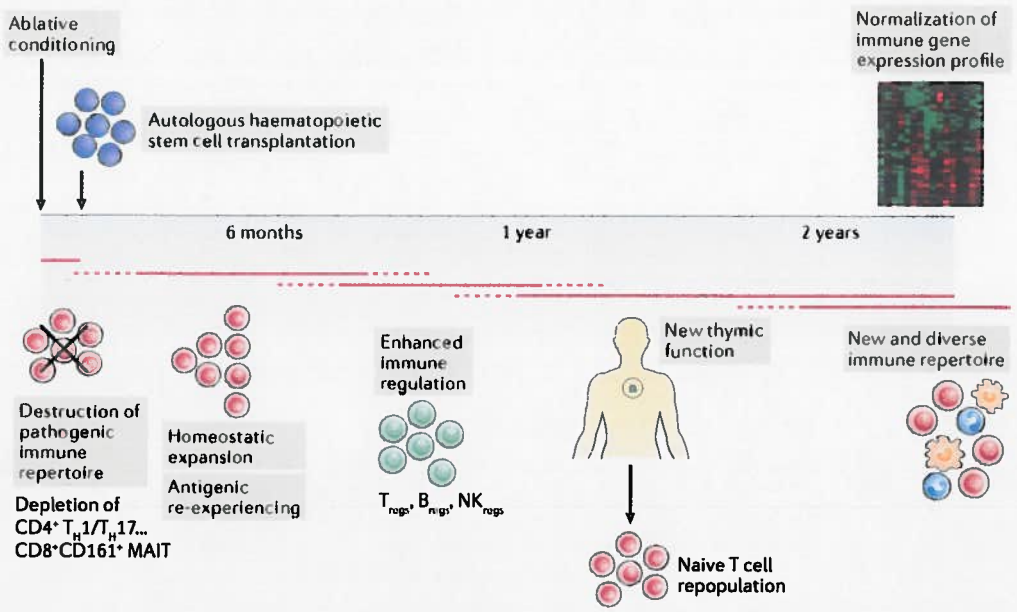

Figure 2 | **Proposed model of therapeutic mechanisms of AHSCT.** Ablative conditioning leads to radical depletion of pathogenic immune cells. During the 6 months after autologous haematopoietic stem cell transplantation (AHSCT), homeostatic expansion of the T cell repertoire produces $CD8^+$ and, in smaller numbers, $CD4^+$ T cells, and antigens are encountered through infection and reimmunization. These processes are associated with potentiation of immune regulation. Subsequently, and most effectively at 1–2 years after transplantation, immune renewal via thymopoiesis leads to increased numbers of naive $CD4^+$ and $CD8^+$ T cells and of $CD31^+$ and signal joint T cell receptor excision circle positive ($sjTREC^+$) recent thymic emigrants, which results in diversification of the T cell repertoire. In parallel, naive B cell reconstitution possibly restores the B cell repertoire and antibody diversity. Some normalization of gene expression profiles that favour restoration of tolerance has been demonstrated after completion of immune reconstitution at 2 years after AHSCT. Further work is required to demonstrate which immune changes are essential for the efficacy of AHSCT in suppressing the inflammatory disease activity in multiple sclerosis. MAIT, mucosal associated invariant T cell; NK, natural killer.

except long-lived plasma cells[39]. During development of the post-AHSCT B cell repertoire, naive and memory B cell subtypes are reconstituted[40], although data on their functional phenotypes are lacking. In systemic lupus erythematosus (SLE), AHSCT reduces or eliminates anti-DNA antibodies, normalizes B cell homeostasis and induces recovery of B cell numbers[41]. These data are consistent with the demonstrated development of a diverse and adult-like immunoglobulin repertoire beyond 4 months after AHSCT[42]. Whether these findings are the same in MS is currently unclear, but the persistence of oligoclonal bands in one study suggests that immunoglobulin-producing cells in the CNS compartment are insufficiently ablated[43].

Gene expression. Gene expression and regulation by microRNAs (miRNAs) in the newly emerging immune repertoire have been studied to gain insight into the mechanisms of immune reconstitution. Comparison of gene expression profiles of $CD4^+$ and $CD8^+$ T cells from patients with MS before and after AHSCT has demonstrated normalization of the profiles: in $CD8^+$ cells, the profiles were more similar to those of cells from unrelated healthy controls than to those of patient cells from before treatment or early after treatment[44]. In another study, expression of the miRNAs miR-16, miR-155 and miR-142-3P, which regulate T cell activation and are aberrantly expressed in MS[45–47], normalized after AHSCT; expression of their putative target genes — *FOXP3*, *FOXO1*, *PDCD1* and *IRF2BP2* — increased, as expected[38]. These data agree with previous findings that the gene and miRNA expression signatures of $CD34^+$ HSCs of patients with MS is not different from healthy controls, indicating that these cells do not have a preprogrammed pro-inflammatory state[48].

Clinical use of AHSCT

Efficacy

Besides isolated reports of AHSCT being used for early treatment of highly aggressive relapsing inflammatory forms of MS[49,50], the initial clinical studies of AHSCT in MS were conducted almost exclusively in patients with high levels of disability and progressive disease[51–54]. Accordingly, in a retrospective long-term analysis published in 2017 (REF. 55), 78% of 281 patients with MS who underwent AHSCT between 1996 and 2005 had progressive MS (66% had secondary progressive MS) and the median Expanded Disability Status Scale (EDSS) score before the procedure was 6.5. In this study, a younger age, RRMS and fewer prior disease-modifying treatments were associated with better neurological outcomes. Consequently, subsequent studies of AHSCT have predominantly included patients with RRMS and an aggressive disease course[9–11,56].

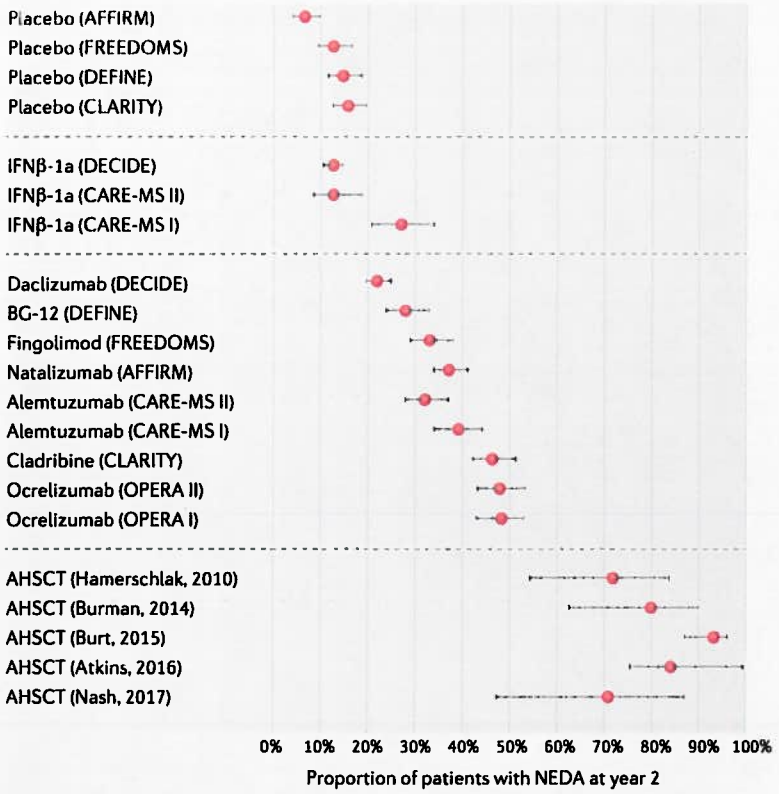

Figure 3 | **Proportion of patients for whom NEDA was achieved at 2 years with disease-modifying therapies and AHSCT.** Red dots are the mean, bars represent 95% confidence intervals. The disease-modifying therapies have been approved or have been effective in phase III trials. Although studies included different patient populations, higher rates of no evidence of disease activity (NEDA) were achieved with autologous haematopoietic stem cell transplantation (AHSCT) than with any other disease-modifying therapy, including those that are considered to have high efficacy. The findings suggest that AHSCT has a more profound effect on disease activity than do current disease-modifying therapies.

Almost all studies of AHSCT in MS have been observational cohort studies in which the efficacy was evaluated by comparing disease activity before and after transplantation. Although this approach generates uncontrolled evidence, it is justified in studies of patients who have an overwhelmingly active or rapidly progressive course that has not responded to appropriate disease-modifying therapy, as randomized controlled studies with these patients would be unethical. Suppression or clear stabilization of such aggressive disease activity after AHSCT can reasonably be regarded as a consequence of the procedure.

One comparative phase II randomized clinical trial of AHSCT has been published — the ASTIMS trial[12] — in which the effect of the treatment was compared with that of mitoxantrone in patients with aggressive RRMS or secondary progressive MS. The number of participants was low ($n=21$), some data were missing and the methodology had other limitations, partly as a result of the purely academic development of the study and the limited financial support; nevertheless, the number of new T2 lesions that occurred over 4 years (the primary endpoint of the study) was 79% lower among patients who underwent AHSCT than among those who received mitoxantrone ($P<0.001$). The proportion of patients in the ASTIMS trial whose disability progressed after AHSCT was 33% at 2 years and 57% at 4 years, but most participants (67%) had secondary progressive disease at baseline. Many participants of other studies that have involved patients with progressive disease[51,54] have also continued to lose neurological function, which has been interpreted as the result of unaffected neurodegenerative processes such as axonal injury and neuronal loss. In the HALT-MS study, in which only patients with RRMS were enrolled, the proportion of patients with disability progression after AHSCT was <10% at 2 years and 5 years[9].

Further studies of AHSCT strengthened the evidence that the therapy is most successful if performed in the earlier inflammatory stages of MS[9,11,14], when profound effects are seen on MRI measures[2] and relapse activity[10]. A study published in 2016 demonstrated that complete suppression of inflammatory activity could be achieved with AHSCT[13]. For the 24 patients enrolled in this study, the mean number of relapses between diagnosis and AHSCT was 1.2 per year, and the mean number of Gadolinium-enhancing lesions seen on the baseline MRI scan (taken in the months preceding the start of the procedure) was 3.9. After AHSCT, no clinical relapses occurred in any of the 23 surviving patients during up to 13 years of follow-up, and none of 327 post-transplantation MRI scans showed Gadolinium-enhancing lesions.

In the absence of direct comparisons between the efficacy of AHSCT and that of approved disease-modifying therapies, some insight can be gained by considering — with caution — the degree of control over disease activity that has been achieved in clinical trials of AHSCT and of treatments that have been licensed on the basis of their efficacy in trials. The level of control is indicated by the proportion of patients who achieve no evidence of disease activity (NEDA), a composite endpoint (no relapses, no disability progression and no MRI activity) that is often reported at 2 years in contemporary trials and has been proposed as a treatment goal in patients with RRMS[57]. Five trials of AHSCT published since 2010 have reported the proportion of patients with RRMS for whom NEDA was achieved at 2 years after transplantation[9–11,13,14,53], and these reports have enabled comparisons with trials of other disease-modifying therapies that are either approved or close to approval[58]. A cross-sectional analysis reveals that the proportion for whom NEDA was achieved at 2 years was 7–16% among those who received placebo, 13–27% among patients who received IFNβ-1a, and 22–48% among patients who received other active drugs; among patients who underwent AHSCT, the proportion was considerably higher, at 70–92% (FIG. 3). Moreover, in trials of drugs, NEDA status was more frequently lost owing to new inflammatory activity, whereas disease activity after transplantation is mainly accounted for by disability progression, especially in studies that included patients with progressive disease[12,51,54].

Table 1 | **Characteristics of patients treated with AHSCT reported during 2014–2017**

Study	Median age (years)	Median EDSS score at baseline (range)	Patients with RRMS (%)	Median MS duration (years)	Mean relapse rate in previous year	Median number of previous treatments	Refs
Burman *et al.*, 2014	31.0	6.0 (1.0–8.5)	85	6.3	4.1	2	10
Burt *et al.*, 2015	37.0	4.0 (3.0–5.5)	81	5.1	>1.5	3	11
Mancardi *et al.*, 2015	36.0	6.5 (5.5–6.5)	22	10.5	1.3	2	12
Currò *et al.*, 2015	28.0	6.0 (5.0–7.0)	100	6.5	2.4	Not reported	56
Muraro *et al.*, 2017	37.0	6.5 (1.5–9.0)	22	6.8	Not reported	2	55
Shevchenko *et al.*, 2015	34.6	3.5 (1.5–8.5)	46	5.0	Not reported	Not reported	71
Atkins *et al.*, 2016	34.0	5.0 (3.0–6.0)	50	6.5	1.2	2	13
Nash *et al.*, 2017	37.0	4.5 (3.0–5.5)	100	5.0	Not reported	3	14

Papers included are those that reported sufficient information about most variables we considered. AHSCT, autologous haematopoietic stem cell transplantation; EDSS, Expanded Disability Status Scale; MS, multiple sclerosis; RRMS, relapsing–remitting MS.

As noted[58], these comparisons must be made with caution because the patient populations and follow-up schedules differed between trials. Nevertheless, the difference in the achievement of NEDA with AHSCT and drug therapies cannot be explained by differences in the populations alone, particularly because participants of AHSCT trials had more aggressive disease than the participants of all the other clinical trials[59]. This severity of disease is illustrated by the characteristics of patients included in AHSCT studies published in the past 2 years (TABLE 1). For example, in comparison with the CARE MS-II trial[60] — the only trial of a currently approved therapeutic that required one prior treatment failure for patient eligibility and consequently included a similar population to those in studies of AHSCT — the median EDSS score is 3.5–6 in AHSCT trials, versus 2.5 in the CARE MS-II trial. Similarly, the median number of previous treatments was two or three in AHSCT trials versus one in CARE MS-II, and the disease duration was 5–10.5 years versus 4.5 years, respectively, all factors that would predict a lower rate of NEDA in the AHSCT trials than in the CARE MS-II trial. However, the mean age was comparable between the trials: 28–38 years versus 35 years.

Some evidence suggests that AHSCT can not only reduce disease activity, but also improve neurological function[4,9–14,55]. In one large single-centre case series ($n = 145$), disability scores had improved at 2 years after AHSCT in one-third of patients[11], predominantly those with RRMS and mild to moderate disability. Work by the same researchers showed that AHSCT failed to improve neurological function and stop worsening in patients with high levels of established disability as a result of progressive MS[31]. This dichotomy is demonstrated on a larger scale by a retrospective long-term analysis that demonstrated significant improvements in neurological function during the first year after AHSCT in patients with RRMS but not those with progressive MS (median EDSS score change 1 year after transplantation: −0.76 versus −0.14, $P<0.001$)[61].

Antithymocyte globulin
A T-cell-depleting polyclonal immunoglobulin from horse or rabbit.

Safety and tolerability

The risks and adverse effects of AHSCT are influenced by the intensity of the procedure; the intensity of the conditioning regimen has a dominant role. The clinical condition of the patient, their age and the presence of comorbidities are also important.

Intensity of AHSCT. The overall intensity of AHSCT can vary widely, and is determined by four main variables: whether chemotherapy is administered for HSC mobilization; whether the haematopoietic graft is manipulated to enrich HSCs (CD34 selection) and remove immune cells (*ex vivo* T cell depletion); the intensity of the conditioning regimen, which depends on the type and dose of the agents used; and whether *in vivo* lymphodepleting serotherapy, such as antilymphocyte or antithymocyte globulin (ATG), is administered. Working definitions that evolved around haematopoietic transplantation for the treatment of cancer are used to distinguish between myeloablative conditioning, reduced-intensity conditioning and non-myeloablative conditioning on the basis of the duration of cytopaenia and the requirement for HSC support[62]. These designations have also been used by some clinicians to qualify different conditioning regimens for autoimmune disease, and have been popular in discussions on social media among people with MS. In transplantation for autoimmune disease, conditioning regimens are classified and defined in the European Society for Blood and Marrow Transplantation (EBMT) guidelines[63] as high-intensity, intermediate-intensity and low-intensity. In a meta-analysis of AHSCT for MS, the reported conditioning regimens were classified according to the EBMT guidelines[64]. High-intensity regimens, which usually include the use of high-dose busulfan or total body irradiation combined with cyclophosphamide and *in vivo* and *ex vivo* T cell depletion, were more frequently used in earlier trials[31,32,51,54,65], partly as a result of sporadic reports of AHSCT for autoimmune disease in which less intense protocols were used and were followed by a high incidence of relapses[66]. Neither a retrospective registry study[61] nor a literature meta-analysis[67], however, documented any advantages of high-intensity regimens over low-intensity regimens in terms of progression-free survival, at least in secondary progressive MS. In addition, life-threatening infections were reported in studies

Uhtoff phenomenon
The recurrence or worsening of pre-existing neurological symptoms, usually transient, experienced by patients with MS after exposure to internal (fever) or external (heat) high temperatures

in which high-intensity conditioning regimens were used[54,68]. Partly on the basis of these concerns, low-intensity regimens that involve administration of cyclophosphamide and ATG — also called nonmyeloablative regimens[62] — were introduced, enabling toxicity to be reduced, which increased confidence in offering AHSCT earlier in the disease course, before the accumulation of irreversible disability in RRMS[4,11].

An intermediate-intensity conditioning regimen called BEAM, which involves a combination of the chemotherapeutics bis-chloroethylnitrosourea (BCNU), etoposide, cytosine arabinoside (ARA-C), and melphalan, has been the most frequently used protocol in Europe (TABLE 2), and has been used in American[9,14,53] and Asian[69] trials. Other intermediate-intensity, BEAM-modified regimens have also been used[70,71]. The different protocols that have been used in the treatment of MS are expected to produce different lymphoablative and myeloablative effects (FIG. 4). Differences between the populations treated with different regimens mean that a reliable comparison of the risk–efficacy ratios between low-intensity and intermediate-intensity regimens cannot be made.

Adverse effects. AHSCT primarily targets the immune system, and immune suppression is, therefore, a necessary and expected (that is, on-target) effect of the procedure. In an analysis of 169 patients, 79% of the early non-neurological adverse effects of AHSCT were secondary to the immunosuppression, and included neutropaenic fever, sepsis, urinary infections and viral reactivations[61].

AHSCT can also have several off-target adverse effects. Neurological toxicity was reported in 26 of 149 (17%) evaluable patients in one study, and occurred within 60 days of transplantation[61]. Transient alopaecia and amenorrhoea are common adverse effects. A small study of patients who underwent low-intensity or intermediate-intensity regimens demonstrated recovery of the menstrual cycle in all patients who were aged ≤32 years, but recovery of menstruation after treatment was reported up to age 41 years[72]. Permanent infertility is a risk, but in a retrospective study of 324 women who underwent HSC transplantation for autoimmune disease, 15 pregnancies were reported, and no congenital, developmental or other disease was reported in the children[73]. Patients with MS who undergo AHSCT can experience disease-specific adverse effects, such as frequent urinary tract infection, the Uhtoff phenomenon, limb spasticity and reduced mobility, all of which can increase the risk of complications and require expert management[74].

Late effects, which are considered to be adverse events that arise months or years after completion of the procedure but might be related to it, are less common, but include secondary autoimmune disease, mostly thyroiditis. In two EBMT Registry analyses published in 2006 and 2011, the incidence of secondary autoimmune disease was 3.6% and 6.4%, respectively[61,75]. A similar incidence was reported in a study published in 2017 that included patients from the EBMT and the Center for International Blood & Marrow Transplant Research (CIBMTR) databases: new autoimmune disease occurred in 14 of 281 patients (5%) over a median follow-up of 6.6 years after AHSCT that followed failure of standard immunomodulatory and immunosuppressive therapies (two or more previous therapies in ~70% of patients)[55]. In the same study, other late adverse events included malignancies in nine patients (3.2%)[55]. Multiple factors contribute to an individual's risk of developing cancer, and the ability to estimate the additional risk as a result of AHSCT remains elusive. Further detail about the complications of AHSCT for the treatment of autoimmune disease is available elsewhere[76].

Treatment-related mortality. Mortality is the main concern that has limited the development and use of AHSCT. In the EBMT Registry, the overall treatment-related mortality among patients with MS who received AHSCT is 2.0% of 829 evaluable patients (as of May 2017, with incomplete data for 2016). This number still incorporates high treatment-related mortality of 7.3% from the earliest use of the treatment during 1995–2000; marked decreases have been reported since, with treatment-related mortality of 1.3% during 2001–2007 (REF. 77), further decreasing to 0.7% (4 of 565) during 2008–2016 and down to 0.2% (1 of 439) in the past 5 calendar years (FIG. 5).

Similarly, a meta-analysis of 15 published studies (which included non EBMT-registered cases) revealed treatment-related mortality of 0.3% among the 349 patients included in seven studies in which the estimated year of transplantation was after 2005 — a marked reduction from the mortality of 3.6% among the 415 patients treated in the older studies[64]. The same meta-analysis identified an inverse relationship between treatment-related mortality and the proportion of patients with RRMS (rather than progressive MS)[64]. A retrospective long-term analysis of 281 patients with MS who underwent AHSCT during 1996–2005 produced similar findings: treatment-related mortality was 2.8%[61], a higher EDSS score was significantly associated with a higher risk of death from any cause (treatment-related and not treatment-related), and progressive MS and high-intensity conditioning regimens were over-represented.

Table 2 | Conditioning regimens for AHSCT reported to the EBMT Registry

Conditioning regimen	No. of patients	% of total patients
Cyclophosphamide + thiotepa	3	0.4
Busulphan + antithymocyte globulin	11	1.5
BEAM ± antithymocyte globulin	441	58.9
Cyclophosphamide + antithymocyte globulin	171	22.8
Other	52	6.9
No information on conditioning regimen	71	9.5
Total	749	100

Data as of March 2016. AHSCT, autologous haematopoietic stem cell transplantation; BEAM, bis-chloroethylnitrosourea, etoposide, cytosine arabinoside and melphalan; EBMT, European Bone Marrow Transplantation Society.

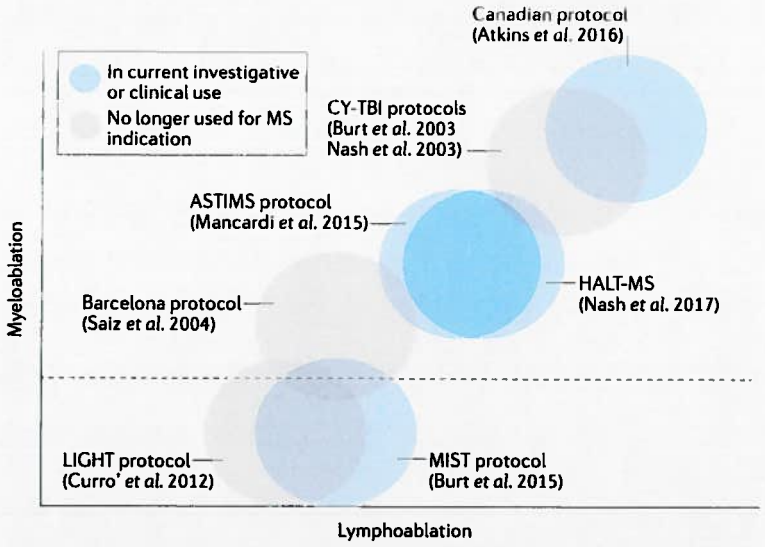

Figure 4 | **Estimated lymphoablative and myeloablative effects of AHSCT protocols for multiple sclerosis.** The expected relative lymphoablative and myeloablative effects in different conditioning protocols used in autologous haematopoietic stem cell transplantation (AHSCT) are depicted. Quantitative metrics are unavailable, and the size of the circles indicates the error in the estimates and the variability of the effects attainable in different patients. The dotted line represents the threshold of the myeloablative effect, above which haematopoietic stem cell (HSC) support is required for haematopoietic recovery and patient survival; in most patients treated with protocols below this threshold, haematopoiesis can recover without HSC transplantation, albeit with a longer recovery interval. These protocols are, therefore, considered non-myeloablative[68].

In combination, these findings indicate that improved patient selection — treatment of patients with RRMS and low EDSS scores — and decreased use of high-intensity conditioning regimens underlie the reduction in treatment-related mortality. Improved supportive care might also be an important factor.

Optimization and recommendations

Studies of AHSCT for the treatment of MS have used a variety of designs in different patient populations (TABLE 3). The experience gained from these studies enables us to develop recommendations to optimize patient selection and methodology.

Patient selection

The opportunity for a patient to benefit from AHSCT depends mainly on their clinical status, supporting the hypothesis that a clear therapeutic window exists during the course of MS[78]. Given the risk and adverse effects of AHSCT, the treatment cannot currently be recommended for the general population of patients with MS. Nevertheless, AHSCT has been designated as a clinical option in the EBMT guidelines for the treatment of patients with aggressive MS that is unresponsive to conventional and approved therapies[63]. However, a critical point for patients and clinicians to understand is that AHSCT is not a neuroregenerative treatment or a treatment that should be used as a last resort after failure of all available treatments. Rather, the optimal timing is immediately after failure of licensed treatment, when the aggressive clinical course of the disease is clear but the patient remains minimally compromised. The current evidence enables us to refine the profile of the ideal candidate for AHSCT on the basis of several important factors: relapses and the phase of the disease, MRI activity, age and disease duration, neurological disability, comorbidities, cognitive impairment and response to prior treatments (BOX 2).

Relapses and phase of disease. Although initial studies of AHSCT in MS predominantly included patients with progressive disease, a profound effect on relapses was soon documented in patients with RRMS[33] and confirmed to varying degrees in all subsequent studies; one study demonstrated total long-term suppression of relapses[13]. A large body of evidence, therefore, supports the general consensus that the patient who would benefit most from AHSCT is still in the relapsing–remitting phase of the disease with inflammatory clinical activity (that is, relapses that can be highly effectively suppressed by the treatment).

Some studies have indicated favourable outcomes of AHSCT in progressive forms of MS[69–71,79]. Following the 2013 revision of the various clinical courses of MS[80], the fact that some patients with progressive MS experience clinical activity (relapses) and/or exhibit inflammatory activity on MRI (Gadolinium–enhancing lesions or new or enlarging T2-positive lesions) is more widely recognized. In these patients, the inflammatory activity might be effectively targeted with AHSCT, as it has been with the immunosuppressants rituximab[81] and ocrelizumab[82]. However, rigorous evidence that AHSCT improves outcomes in patients with progressive MS, even with inflammatory activity, is lacking.

MRI markers of activity. Gadolinium-enhancing lesions and new or enlarging T2 lesions are well-established indices of inflammatory MS activity. It has been clear since 2001 that AHSCT can completely suppress inflammatory lesion activity detectable with Gadolinium-enhancing MRI for at least 36 months[2]. This abrogation of MRI activity has been replicated over a follow-up period of 6–7 years[13]. Nevertheless, a complete absence of MRI activity after transplantation has not been observed in every study. In one study in which a cyclophosphamide-based low–intensity conditioning regimen was used, reoccurrence of MRI inflammatory activity was frequently seen 6–12 months after AHSCT[56]. In several studies of patients who were treated with intermediate-intensity regimes, such as BEAM, 8–10% of individuals exhibited new or active lesions on MRI scans at 2–5 years after treatment[9,10,14,79].

In two reports, progression-free survival was significantly better in patients with MRI lesion activity at baseline than in those without[10,79]. The evidence from these studies, together with the need to demonstrate active inflammation to advocate use of a treatment strategy that targets inflammation, justify recent (within the previous 12 months) MRI inflammatory activity as a key requirement in patient selection for AHSCT.

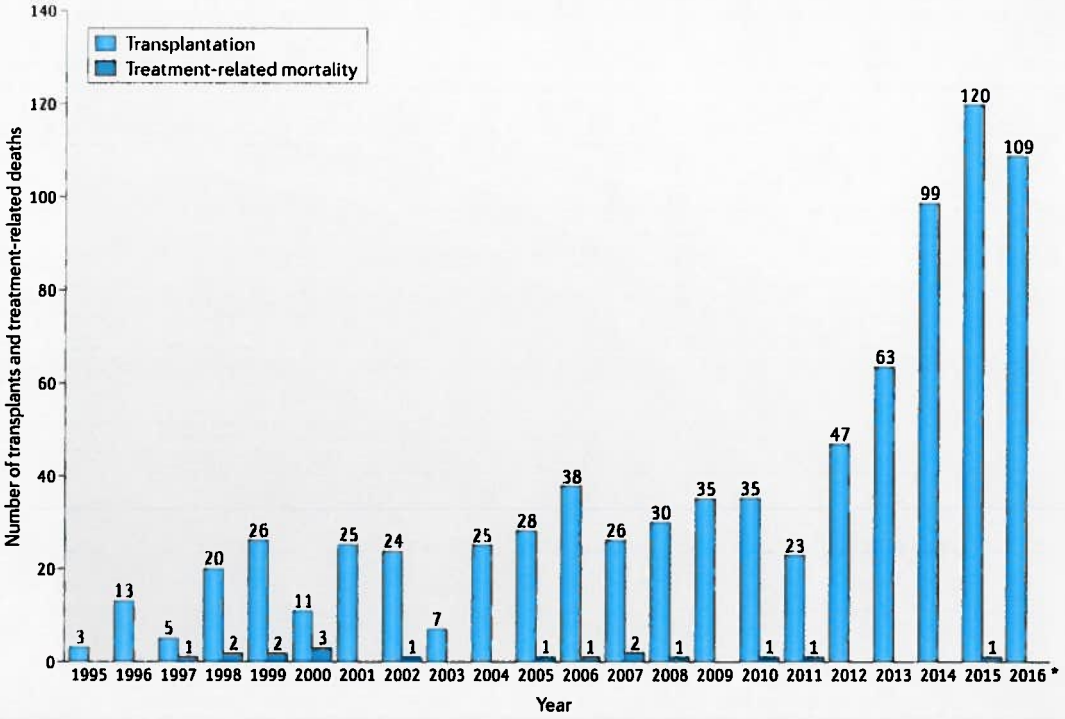

Figure 5 | **Number of AHSCT procedures for treatment of multiple sclerosis and treatment-related mortality.** Data as reported to the European Blood and Marrow Transplantation Society (EBMT). The overall (1995–2016) treatment-related mortality for autologous haematopoietic stem cell transplantation (AHSCT) is 2.0%. However, treatment-related mortality has decreased over time and was 0.2% during the past 5 years (2012–2016). *Data from 2016 are incomplete, and data from 2017 are not yet available.

Age and duration of disease. In one large case series, patients aged <40 years who underwent AHSCT within 5 years of disease onset had better progression-free survival than older patients with longstanding disease[61]. The onset of progressive disease is age-dependent, and usually occurs at age 40–50 years; the frequency of relapses decreases with increasing age[83,84]. In a long-term analysis, age was identified (together with progressive MS and number of prior immune-modifying therapies) as an independent factor associated with neurological progression-free survival[61]. The duration of clinical disease strongly correlates with age, so a shorter disease duration is expected to be associated with a higher probability of active inflammatory disease. Consequently, although older age alone does not negatively affect overall survival after AHSCT for lymphoma[85], in the treatment of MS, the evidence provides the strongest rationale for AHSCT in young patients (aged <45 years) with a short disease duration (<10 years).

Neurological disability. In studies of AHSCT conducted before 2005, the baseline disability of patients was usually high: one study of long-term outcomes[61] showed that the median EDSS score was 6.5. In this study, a higher EDSS score was significantly associated with poorer overall survival (HR 2.03 per EDSS point). Similarly, in a meta-analysis, a baseline EDSS score >6 was significantly associated with a higher treatment-related mortality ($P=0.013$)[64]. These associations can be explained by a high incidence of comorbidities (such as urinary tract infections and chronic lung disease) and their attendant complications in non-ambulatory patients[86], as well as an increased risk of death from progression of MS. Partly on the basis that higher EDSS scores are frequently associated with progressive MS than with RRMS, AHSCT studies from the past few years have excluded patients with severe disability by, for example, limiting baseline EDSS scores to 5.5 (REFS 9,11,14).

In rare cases of extremely aggressive MS that causes a high degree of disability in a matter of weeks, AHSCT can be considered as a potentially lifesaving treatment and has been used successfully in this context[49,50,87,88]. However, besides these situations, patients with an EDSS score <6 (ambulatory without aid) are the most appropriate candidates for AHSCT and — on the basis of published evidence[61,64] and our clinical experience — we suggest that patients with established EDSS scores ≥7 are at high risk of complications and treatment failure, so are not appropriate candidates for AHSCT.

Previous response to MS therapies. In the vast majority of cases, AHSCT has been offered as part of a clinical study or as compassionate therapy after failure of one or more disease-modifying therapies. Given the risks and adverse effects, treatment with AHSCT outside of clinical trials seems reasonable and ethical only after failure of approved therapy. In line with contemporary views on the management of RRMS[89,90], we suggest that failure

Table 3 | **Study design and outcomes in recent clinical studies of AHSCT for MS**

Study	Patient sample size	Median follow-up (months)	Regimen type	Patients with disability progression at 2 years (%)	Patients with disability progression at 5 years (%)	Refs
Burman *et al.*, 2014	41	47.4	Intermediate	10	23	10
Burt *et al.*, 2015	145	24.0	Low	7	13	11
Mancardi *et al.*, 2015	9	48.0	Intermediate	33	Not reported	12
Currò *et al.*, 2015	7	60.0	Low	14	43	56
Muraro *et al.*, 2017	281	79.2	Mixed	16	54	55
Shevchenko *et al.*, 2015	99	48.9	Intermediate	1	13	71
Atkins *et al.*, 2016	24	80.4	Intermediate	30	30	13
Nash *et al.*, 2017	25	62.0	Intermediate	10	14	14

Papers included are those that reported sufficient information about most variables we considered. AHSCT, autologous haematopoietic stem cell transplantation; MS, multiple sclerosis.

of one licensed disease-modifying drug of high efficacy (defined as Category 2 (REF. 91), which currently includes alemtuzumab or natalizumab, but is likely to include rituximab and ocrelizumab once these therapies are approved — ocrelizumab is approved in the USA, but not yet in Europe), owing to a demonstrated lack of efficacy, is sufficient to consider offering AHSCT to otherwise clinically appropriate patients with aggressive RRMS.

For patients with average active MS (which is distinct from aggressive), the current prevailing opinion is to initiate treatment with a first-line therapy, followed by escalation to natalizumab, fingolimod or alemtuzumab in those who fail to respond[89]. In our opinion, escalation through two lines of therapy before considering AHSCT is acceptable but not required: patients who experience persistence of their disease or breakthrough of substantial clinical and MRI inflammatory activity during induction treatment with a high-efficacy monoclonal antibody (as defined above) administered as a first-line therapy could be considered as candidates for AHSCT, as well as for alternative approved high-efficacy treatment options. However, prior treatment with more than two immunotherapies is associated with poorer progression-free survival after AHSCT[61], highlighting the limited window of opportunity for effective MS treatment, and the fact that treatment escalation over several years precludes the chances of success with any treatment that is given too late. With >10 currently approved drugs for MS, the risk of missing this therapeutic window is of particular concern.

Box 2 | **Profile of an appropriate candidate for AHSCT**

- Relapsing–remitting multiple sclerosis (MS) or progressive MS for a short period of time
- Recent clinical inflammatory activity (i.e. relapses in the previous 12 months)
- Recent MRI inflammatory activity (Gadolinium-enhancing areas or an increase in the number of T2 lesions compared with a recent previous scan)
- Young age (<45 years)
- Short disease duration (no longer than 10 years)
- Low to moderate disability (Expanded Disability Status Scale score lower than 6 or up to 6.5 if the highest score has been reached within the previous few months and the patient has clinical and MRI inflammatory activity
- Failure of approved, high-efficacy disease-modifying therapy (preferably not more than two disease-modifying therapies)
- No substantial comorbidities
- Has the capacity to give informed consent and to adhere to treatments and recommendations for prophylaxis in the immune compromised phases

Comorbidities and cognitive impairment. Systemic and organ-specific comorbidities affect survival after AHSCT[85]. Substantial cardiac, renal, pulmonary or hepatic dysfunction, active infections, or other conditions that could increase the risk of severe complications and mortality, are contraindications for AHSCT[74]. The pre-transplantation workup must always include screening and assessment for such conditions, as detailed elsewhere[63]. Furthermore, adequate cognitive capacity is required to fully understand the possible adverse effects and risks of AHSCT, and to comply with treatments and recommendations, which are important elements in the safety of the procedure.

AHSCT methodology

Increasing clinical experience with AHSCT is enabling refinement of the methodology for the procedure and development of a recommended protocol (BOX 3).

HSC mobilisation. For HSC mobilization, use of high-dose cyclophosphamide with G-CSF is preferable to use of G-CSF alone for several reasons: the HSC yield is better; the induction treatment effect is potentiated by sequential immunosuppression during mobilization and conditioning; the combined treatment does not exacerbate the disease, unlike G-CSF alone; the number of mature immune cells in the graft is lower, and recovery of T cell clones from the autologous graft is poor, which reduces the potential for carryover of disease-mediating T cells with the transplant[92]. We support the mobilization regimen recommended by the EBMT[63]: cyclophosphamide at 2–4 g/m^2 of body surface area with mesna and cautious hyperhydration for bladder protection, followed by 5–10 μg/kg G-CSF daily until the completion of HSC collection.

Conditioning regimen. The optimal intensity of the conditioning regimen remains an open question, given the lack of published comparative data. High-intensity regimens are burdened by a high toxicity profile, as indicated by previous analyses[54,61], although these findings have not been confirmed in a larger study[93]. The regimen that includes high-dose busulfan, described in 2009 (REF. 74) and uniquely used for treatment of MS in the study reported in 2016 (REF. 13), completely suppressed MS inflammatory activity, but caused severe liver toxicity in two patients, which resulted in the death of one. Low-intensity regimens, such as those based on cyclophosphamide, are safer and require less supportive care; however, some data indicate that these regimens are associated with a higher incidence of disease reactivation[4,53]. The conditioning regimens with the strongest traction within each intensity category are cyclophosphamide and ATG (low intensity), BEAM and ATG (intermediate intensity) and cyclophosphamide, busulfan and ATG (high intensity). In the absence of evidence from randomized comparisons, we endorse the preferential use of BEAM and ATG — the scheme that is specifically recommended for the treatment of MS in the EBMT guidelines[63] — by virtue of its extensive track record that indicates good safety and high efficacy[14,79], and of the opportunity it provides for comparisons across data sets.

Autologous HSC enrichment and dose. The advantage of graft purification — with CD34 selection, for example — is unclear. A theoretical advantage is a reduced risk of re-introducing mature leukocytes that might include pathogenic cells. However, the only randomized study in which CD34-selected and CD34-unselected HSC grafts have been compared — conducted in patients with rheumatoid arthritis — showed no difference in outcomes[94]. Similarly, a retrospective analysis of AHSCT for systemic sclerosis failed to demonstrate any benefit of CD34 selection on patient outcomes[95]. Evidence in MS is limited to a small study of patients with progressive MS, but this work also demonstrated no obvious advantage of CD34 selection[96]. These consistently negative findings have weakened support for the use of CD34 selection[63], especially when HSC mobilization is carried out with the use of high-dose cyclophosphamide with G-CSF rather than with G-CSF alone.

Box 3 | **Recommended AHSCT methodology for treatment of MS**

Mobilization
Cyclophosphamide at 2–4 g/m^2 body surface area with mesna and hyperhydration, followed by 5–10 μg/kg granulocyte colony-stimulating factor (G-CSF) daily

Immunoablative conditioning
BEAM (bis-chloroethylnitrosourea, etoposide, cytosine arabinoside and melphalan)

Autograft
Unselected peripheral blood product: minimum dose of 2×10^6 $CD34^+$ cells/kg, and preferably $\geq 5 \times 10^6$ $CD34^+$ cells/kg

Serotherapy for *in vivo* T cell-depletion
Antithymocyte globulin (ATG) from horse or rabbit, alongside administration of corticosteroids to attenuate adverse effects

Irrespective of graft manipulation, we support the EBMT recommendation for transplantation that a minimum dose of 2×10^6 $CD34^+$ cells/kg should be reinfused[63]. However, evidence is accumulating from treatment of haematological malignancies that higher doses of HSC can promote faster platelet engraftment and are associated with better overall survival[97,98,99]. Consequently, a dose of 5×10^6 $CD34^+$ cells/kg is currently defined as optimal[100,101].

Serotherapy. Use of serotherapy, such as antilymphocyte or ATG, administered with or immediately following the conditioning regimen complements the immunosuppressive effect of conditioning, as the resulting T cell depletion can be critical in preventing the engraftment of any T cells that are reinfused; this effect is expected to be especially important with an unselected HSC graft. In addition, ATG has known immunomodulatory effects, including induction of adaptive regulatory T cells[102], that might influence the earliest, and perhaps most critical, stages of immune reconstitution.

Antimicrobial prophylaxis and monitoring. General guidelines for prevention and management of infections in patients undergoing haematopoietic stem cell transplantation are available[103,104]. Additionally, in patients with MS, monitoring of cytomegalovirus and Epstein–Barr virus viraemia in the 3 months after transplantation is recommended, and might require a pre-emptive treatment in case of increasing viral load.

This recommendation is mostly based on the experience in allogeneic transplantation[103,105], which results in a higher degree of post-transplantation immunosuppression than autologous transplantation; however, administration of ATG in autologous transplantation, commonly done in protocols for treatment of patients with MS, can increase the risk of viral reactivation[104].

Conclusions

AHSCT provides a unique approach to the treatment of MS. Unlike current disease-modifying therapies that either modulate or partially suppress the immune system, AHSCT relies on ablation of the immune and, to a variable extent, myeloid systems, followed by reconstitution of a profoundly modified immune system, in a process with characteristics of immune 'resetting'. This approach is associated with risks that are generally greater than those associated with disease-modifying therapies, but predominantly occur early in the treatment procedure, whereas the risks of chronic or cyclic immune modulation or suppression, although initially low, increase over time. Furthermore, evidence from trials suggests that AHSCT is considerably more effective than current disease-modifying therapies at arresting inflammatory activity in MS.

The patients who are most likely to benefit from AHSCT are those with RRMS, a high frequency of relapses, MRI markers of inflammatory activity, a young age, a short duration of disease and limited disability, and who have been referred soon after a highly active MS therapy has failed and who are not affected by substantial comorbidities and cognitive impairment. As is

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Box 4 | Challenges preventing randomized controlled trials of AHSCT for MS

Lack of funding

- Autologous haematopoietic stem cell transplantation (AHSCT) does not rely on proprietary new therapeutics and has not benefited from pharmaceutical industry support
- Competition for time, patients and resources with trials that are well supported by the pharmaceutical industry
- Ever-increasing regulatory and administrative burden, which make low-funded academic trials particularly unsustainable

Clinician-related factors

- Safety concerns rooted in early data on transplant-related mortality (5–10%) and not updated to current figures (0.3%)
- Competing interests related to support for research and clinical service development and/or personal remuneration from the pharmaceutical industry that reduce incentive to develop AHSCT[113]
- A perception that AHSCT is not an elegant or 'clever' selective immune intervention
- Unwillingness to collaborate with or rely on other specialists for the administration of treatment for multiple sclerosis (MS)

Patient-related factors

- Low acceptance of randomization to control therapies from patients who are entering a trial and want AHSCT

Study design difficulties

- Impossible double (patient) blinding
- Continuous introduction of new and increasingly effective therapies for MS over past 15 years

the case for disease-modifying therapies, beneficial effects of AHSCT on neurological progression in MS are plausible, but cannot be reliably demonstrated without well-conducted, long-term randomized controlled trials.

To date, a lack of support from the pharmaceutical industry, among other factors (BOX 4), has slowed the development of AHSCT. Yet, the current evidence from studies of AHSCT in MS makes a strong case to support the need for clinical trials, firstly to establish the safety, efficacy and cost-effectiveness of AHSCT in comparison with disease-modifying therapies in patients with highly active RRMS, and secondly to assess whether AHSCT might have a place in the treatment of early-stage forms of progressive MS with inflammatory activity. Indeed, preliminary evaluations suggest that the treatment could be cost-effective[106] and offer savings to patients and/or health authorities when compared with the cost of current biologics.

Trials are needed to establish whether AHSCT could be recommended for the treatment of patients with inflammatory activity who have not tried high-efficacy disease-modifying therapies, but we believe that enough evidence already exists to support the use of AHSCT for treatment of patients with aggressive RRMS and those with active RRMS in whom high-potency, approved, disease-modifying therapy has failed because of a lack of efficacy. Indeed, in December 2016, the Swedish Board of Health and Welfare included AHSCT as an option alongside fingolimod and alemtuzumab (as well as natalizumab for people with a negative JC virus antibody test or with low antibody levels) in their treatment recommendations for active RRMS[107]. We advocate healthcare organizations in all other countries to consider introducing AHSCT as the standard of care for these indications, and to regularly reassess and update their guidelines on the basis of new evidence that could alter the indications.

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